Toxicity & Teratogenicity Studies in Avian Embryos-**Gum Arabic**-FDA Contract #71-330
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GUM ARABIC

TOXICITY and TERATOGENICITY STUDIES in AVIAN EMBRYOS

FDA CONTRACT #71-330

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GUM ARABIC

TOXICITY and TERATOGENICITY STUDIES in AVIAN EMBRYOS

Gum arabic was administered in water solution to provide maximum dose levels of 400 mg/kg. Dose levels up to 200 mg/kg were non-toxic when injected into the air cell of fertile eggs either prior to incubation or after 96 hours development. A level of 400 mg/kg by air cell administration at 96 hours was toxic and produced a significant chi-square value.

Yolk administration of gum arabic at either of the times employed produced statistically significant increases in mortality at all dose levels tested in the range of 20 to 200 mg/kg.

The low incidences of abnormal embryos observed and the lack of statistical significance in comparison with the control embryos suggests that gum arabic is not teratogenic under the conditions of these studies.

GENERAL PROCEDURES

The protocols as specified under FDA Contract #71-330 were followed in the investigation of toxicity and potential teratogenicity of the specified substance. The toxicity of the substance was evaluated from the percentage hatch of embryos injected either in the air cell or yolk at either zero hours (post-incubation) or after 96 hours incubation to provide four separate evaluations.

EGG SOURCE AND HANDLING

All eggs used in these investigations were from Shaver Starcross pullets housed at the Poultry Research Center of the University of Arizona in Tucson. The parent stock was maintained on the University of Arizona breeder diet which had been formulated to provide more than adequate amounts of all the known nutrients required by the breeding hen.

The feed was specially prepared to assure no contaminations and did not contain any additive drugs such as antibiotics. All eggs prior to use (within 48 hours of lay) were candled to remove any containing blood spots, abnormal air cells or abnormal shells, and only clean eggs ranging in weight from 23 - 26 ounces per dozen were used.

The supply flock was tested to assure the absence of Pullorum and Mycoplasma gallisepticum.

The eggs were incubated in forced draft Jamesway 252 machines with automatic temperature and humidity controls and an automatic turning device.

COMPOUND HANDLING FOR INJECTION

The substance tested was solubilized in a number of the prescribed solvents in order to determine the maximum concentrations which could be employed. Where possible, water was the solvent of choice. Maximum

injection volume was 0.05 ml. and all solvents and glassware were autoclaved prior to preparation of the solutions for use. The dose levels were administered with a microliter syringe using sterilized needles.

The preliminary range-finding studies using each of the administration routes and times were carried out with 10 - 25 eggs per dose level and included solvent controls, untreated controls and either drilled or pierced controls.

The actual dose-response protocol was carried out in two or more injections on different days to produce a minimum of 100 eggs at each dose level in five or more levels selected from the range- finding studies.

EXAMINATIONS OF EMBRYOS AND CHICKS

Eggs were candled daily and the dead embryos removed, examined and any abnormalities recorded. Five chicks from each dose level in each hatch were X-rayed to determine any skeletal abnormalities. Additional eggs injected at the approximate LD-50 level and an additional level below that were incubated and embryos at 8, 14, 17 days and hatch chicks removed for histopathological examinations.

In additional studies representative chicks from the dose-response protocol were saved. These chicks were housed in electrically-heated battery brooders with raised wire floors and fed University of Arizona diets. Feed consumption and growth rates were evaluated at 6 weeks of age and a sample of the birds sacrificed for gross and histopathological examinations.

The remaining birds in each group were maintained to 6 months of age and then sacrificed.

DATA HANDLING

All data were coded on forms provided by FDA for computer input. In addition to summaries of mortalities and abnormalities, a number of statistical evaluations were carried out. These statistical analyses included the following for both mortality and the incidence of abnormal embryos:

1. Chi-square tests for all dose levels and for each level against the solvent control.
2. Linear regression analyses + chi square test of linearity.
   a. % response against dose
   b. % response against log dose
   c. log % response against dose
   d. arcsin transformation against dose
   e. arcsin transformation against log dose
3. Log dose against Probit using Finney's maximum likelihood method.
   a. Where significant, the LD-30, 50, 70 and 90's were estimated with 95% confidence intervals.
4. One-way analyses of variance.
5. Linear regression with replication.

Gum arabic was solubilized in water for use in the test protocols; the maximum dose level was employed with a solution of 200 mg/ml to provide 200 mg/kg (10 mg/egg).

MORTALITY

Mortality data for the individual test protocols are shown in Tables 1 - 4. Air cell administration at zero hours failed to produce an increase in embryo mortality statistically different from that of the water-injected control (Table 1). Chi-square analyses of these data are shown in Table 5. Embryo mortality obtained with gum arabic injections into the air cell after 96 hours incubation produced a significant increase in mortality for only the highest dose level employed (400 mg/kg). The Chi-square value for this dose level in comparison with the solvent control was 5.32 (Table 5).

Yolk administrations at either 0 or 96 hours produced statistically significant increases in embryo mortalities, suggesting that this compound was toxic by this administration group (Tables 3 and 4). Chi-square analyses of the per cent mortality data showed statistical significance ($P < 0.05 - 0.005$) for all dose levels employed; the dose ranged from 20 mg/kg to 200 mg/kg. Linear regression analyses of log dose against probits of per cent mortality values failed to yield statistical significance and, therefore, estimates of LD-50 values could not be determined (Table 8). Gum arabic was toxic to chicken embryos when yolk administration was employed.

TERATOLOGY

The incidence of abnormal embryos in those eggs injected with gum arabic was quite low (Tables 1 - 4). Chi-square analyses of abnormalities and HLSV abnormalities failed to show statistical significance for any of the test protocols employed (Tables 6 & 7).

The specific findings for the teratological examinations are listed in Table 9. The lack of statistical significance by the chi-square tests for the teratology data on eggs injected with gum arabic in comparison with the water injected controls leads to the conclusion that this compound is not teratogenic in the avian embryo under the conditions of these studies.

Post Hatch Data

Chicks hatched from eggs which had been injected with either water or gum arabic (20 or 200 mg/kg) showed no differences in hatch weight (Table 10).

At 6 weeks of age there is a suggestion of smaller body size for the gum arabic treated male birds, but this difference was not apparent at 6 months of age. Feed consumption was not greatly different among the three groups of birds, and age at sexual maturity was essentially unaffected by gum arabic.

TABLE 1

GUM ARABIC in H_2O

Air Cell - 0 Hrs

Dose, ppm	No. Fertile	Mortality %	Mortality #	Abnormal Total %	Abnormal Total #	Abnormal H-S-V-L %	Abnormal H-S-V-L #	Abnormalities by category: Head %	Head #	Skeletal %	Skeletal #	Viscera %	Viscera #	Limbs %	Limbs #	Struc-tural %	Struc-tural #	Toxic Response %	Toxic Response #	Functional %	Functional #
200.0	134	5.97	8	2.98	4	0.74	1	0.74	1												
150.0	105	8.57	9	0.95	1	1.90	2	0.95	1					0.95	1	0.95	1				
100.0	104	10.57	11	1.92	2	0.96	1	0.96	1									0.96	1		
50.0	104	11.53	12	0.00	0	0.00	0														
20.0	105	12.38	13	0.00	0	0.00	0														
0.0	130	9.23	12	0.00	0	0.00	0														
[illegible]rilled	129	10.07	13	0.00	0	0.00	0														
[illegible]ntrol	367	7.35	27	0.27	1	0.00	0											0.27	1		

SUMMARY - ALL DOSE LEVELS

552	9.60	53	1.27	7	0.72	4	0.54	3	0.00	0	0.00	0	0.18	1	0.18	1	0.18	1	0.00	0

TABLE 2

GUM ARABIC in H_2O

Air Cell - 96 hrs

Dose, ppm	No. Fertile	Mortality %	Mortality #	Abnormal Total %	Abnormal Total #	Abnormal H-S-V-L %	Abnormal H-S-V-L #	Abnormalities by category: Head %	Head #	Skeletal %	Skeletal #	Viscera %	Viscera #	Limbs %	Limbs #	Structural %	Structural #	Toxic Response %	Toxic Response #	Functional %	Functional #
200.0	27	14.81	4	0.00	0	0.00	0														
100.0	135	7.40	10	0.00	0	0.00	0														
50.0	72	8.33	6	0.00	0	0.00	0														
00.0	100	9.00	9	1.00	1	1.00	1					1.00	1								
0.0	100	7.00	7	0.00	0	0.00	0														
20.0	99	6.06	6	0.00	0	0.00	0														
0.0	151	2.64	4	0.66	1	0.66	1					0.66	1								
drilled	139	3.59	5	0.00	0	0.00	0														
control	367	7.35	27	0.27	1	0.00	0											0.27	1		

SUMMARY - ALL DOSE LEVELS

533	7.88 42	0.19 1	0.19 1	0.00 0	0.00 0	0.19 1	0.00 0	0.00 0	0.00 0	0.00 0	

TABLE 3

GUM ARABIC in H_2O

Yolk - 0 Hrs

Dose, ppm	No. Fertile	Mortality %	Mortality #	Abnormal Total %	Abnormal Total #	Abnormal H-S-V-L %	Abnormal H-S-V-L #	Abnormalities by category: Head %	Head #	Skeletal %	Skeletal #	Viscera %	Viscera #	Limbs %	Limbs #	Struc-tural %	Struc-tural #	Toxic Response %	Toxic Response #	Functional %	Functional #
200.0	211	73.93	156	0.47	1	0.00	0									0.47	1				
150.0	216	70.37	152	0.46	1	0.46	1	0.46	1												
100.0	215	69.30	149	1.39	3	0.46	1					0.46	1			0.46	1	0.46	1		
50.0	215	63.25	136	1.86	4	0.93	2	0.46	1			0.46	1			1.39	3				
20.0	213	57.74	123	3.28	7	0.93	2	0.46	1			0.46	1			1.87	4	0.46	1		
0.0	235	46.38	109	1.27	3	0.42	1	0.42	1							0.85	2				
pierced	219	55.25	121	1.36	3	0.91	2	0.45	1			0.45	1			0.45	1				
control	367	7.35	27	0.27	1	0.00	0											0.27	1		

SUMMARY - ALL DOSE LEVELS

1070	66.92	716	1.50	16	0.56	6	0.28	3	0.00	0	0.28	3	0.00	0	0.84	9	0.19	2	0.00	0

TABLE 4

GUM ARABIC in H_2O

Yolk - 96 Hrs

Dose, ppm	No. Fertile	Mortality %	Mortality #	Abnormal Total %	Abnormal Total #	Abnormal H-S-V-L %	Abnormal H-S-V-L #	Abnormalities by category: Head %	Head #	Skeletal %	Skeletal #	Viscera %	Viscera #	Limbs %	Limbs #	Structural %	Structural #	Toxic Response %	Toxic Response #	Functional %	Functional #
200.0	180	51.66	93	1.11	2	0.55	1	0.55	1									0.55	1		
150.0	177	65.53	116	0.00	0	0.00	0									0.59	1				
100.0	168	57.73	97	0.59	1	0.00	0					0.56	1								
50.0	178	62.92	112	0.56	1	0.56	1	0.55	1					0.55	1						
20.0	180	52.77	95	0.55	1	1.11	2									0.40	1				
0.0	246	24.79	61	0.40	1	0.00	0											0.27	1		
pierced	163	23.31	38	0.00	0	0.00	0														
control	367	7.35	27	0.27	1	0.00	0														

SUMMARY - ALL DOSE LEVELS

883	58.10	513	0.57	5	0.45	4	0.23	2	0.00	0	0.12	1	0.12	1	0.12	1	0.12	1	0.00	0

TABLE 5

GUM ARABIC
CHI-SQUARE ANALYSES
% MORTALITY

Dose, mg/kg	Air Cell		Yolk	
	0 hrs	96 hrs	0 hrs	96 hrs
20.0	0.32	1.03	5.33*	33.87*
50.0	0.13	1.78	12.22*	60.58*
100.0	0.02	3.73	23.18*	44.52*
150.0	0.00	2.47	25.59*	68.54*
200.0	0.59	2.52	33.86*	31.36*
400.0	-	5.32*	-	-
All Doses (DF)	3.70(5)	8.12(6)	49.98(5)*	95.87(5)*

*Probability < 0.05 - 0.005.

TABLE 6

GUM ARABIC
CHI-SQUARE ANALYSES
ABNORMALITIES

Dose, mg/kg	Air Cell		Yolk	
	0 hrs	96 hrs	0 hrs	96 hrs
20.0	0.00	0.05	1.25	0.25
50.0	0.00	0.04	0.01	0.24
100.0	0.76	0.19	0.09	0.20
150.0	0.01	0.14	0.18	0.03
200.0	2.19	0.00	0.16	0.07
400.0	-	0.95	-	-
All Doses (DF)	9.41(5)	3.70(6)	8.18(5)	2.18(5)

TABLE 7

GUM ARABIC
CHI-SQUARE ANALYSES
HLSV ABNORMALITIES

Dose, mg/kg	Air Cell		Yolk	
	0 hrs	96 hrs	0 hrs	96 hrs
20.0	0.00	0.05	0.01	0.03
50.0	0.00	0.04	0.01	0.03
100.0	0.01	0.19	0.42	0.00
150.0	0.01	0.14	0.42	0.00
200.0	0.00	0.00	0.00	0.03
400.0	-	0.95	-	-
All Doses (DF)	3.06(5)	3.70(6)	2.51(5)	3.30(5)

TABLE 8

GUM ARABIC
PROBIT ANALYSES

Log Dose vs Probit	Air Cell		Yolk	
	0 hrs	96 hrs	0 hrs	96 hrs
1. Mortality	NS	NS	NS	NS
2. Abnormalities	NS	NS	NS	NS

TABLE 3

GUM ARABIC

TERATOGENIC FINDINGS

TREATMENT	TOTAL NO. EXAMINED	TOTAL NO. ABNORMAL	SPECIFIC FINDINGS NO.	SPECIFIC FINDINGS DESCRIPTION
Untreated Controls	367	1	1	bulla back
Drilled Control - 0 hrs	129	0	0	
Drilled Control - 96 hrs	139	0	0	
Pierced Control - 0 hrs	219	3	1	celosomia
			1	anopthalmia-left dysgnathia, beak, brachygnathia -
				mandible
			1	dwarfism
Pierced Control - 96 hrs	163	0	0	
In Water - Air Cell - 0 Hrs				
200.0 mg/kg	134	4	3	granulation tissue - liver
			1	anophthalmia - bilateral, shortened - maxilla
150	105	1	1	brachygnathia - mandible, phocomelia - hind limb, dwarfism
100	104	2	1	bulla - back
			1	anophthalmia - right, dysgnathia - beak
50	104	0	0	
20	105	0	0	
0	130	0	0	

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GUM ARABIC
TERATOGENIC FINDINGS

TREATMENT	TOTAL NO. EXAMINED	TOTAL NO. ABNORMAL	SPECIFIC FINDINGS NO.	DESCRIPTION
In Water - Air Cell - 96 hrs				
400.0 mg/kg	27	0	0	
200	135	0	0	
150	72	0	0	
100	100	1	1	celosomia - abdomen
50	100	0	0	
20	99	0	0	
0	151	1	1	celosomia - abdomen
In Water - Yolk - 0 hrs				
200.0 mg/kg	211	1	1	dwarfism
150	216	1	1	anophthalmia - right, acrania, dysgnathia - beak
100	215	3	1	dwarfism
			1	celosomia - abdomen
			1	hemorrhage - umbilical cord
50	215	4	1	clubbed down, celosomia - abdomen
			2	dwarfism
			1	dysgnathia - beak

Sheet 3 - Table 9

GUM ARABIC

TERATOGENIC FINDINGS

TREATMENT	TOTAL NO. EXAMINED	TOTAL NO. ABNORMAL	SPECIFIC FINDINGS NO.	DESCRIPTION
In Water - Yolk - 0 hrs				
20.0 mg/kg	213	7	4	dwarfism
			1	anopthalmia - left, dysgnathia, beak
			1	celosomia - abdomen
			1	umbilical cord around fetus
0.0	235	3	1	shortened - maxilla, brachygnathia - mandible
			2	dwarfism
In Water - Yolk - 96 hrs				
200.0 mg/kg	180	2	1	hemorrhage - generalized
			1	dysgnathia - beak
150	177	0	0	
100	168	1	1	dwarfism
50	178	1	1	celosomia - abdomen
20	180	1	1	dysgnathia, malrotation - left, ankle
0	246	1	1	dwarfism

TABLE 10

GUM ARABIC
POST HATCH DATA

Incubation Date - 3/6/72

Label	Dose, mg/kg	Age at Sexual Maturity Days	Average Body Wt., gm at Hatch	Average Body Wt., gm 6 wks M	Average Body Wt., gm 6 wks F	Average Body Wt., gm 6 ~~wks~~ mos M	Average Body Wt., gm 6 ~~wks~~ mos F	Average Feed Cons./bird 6 wk, gm	Average Feed Cons./bird 6 ~~wks~~ mos ~~gm~~ kg
221	Water	140	35.9	502	419	1789	1503	979	8.06
222	20.0	142	34.6	438	388	1902	1666	885	8.43
226	200.0	147	34.7	458	426	1703	1552	911	8.35